The Effects of Methylmercury on Isolated Cardiac Tissues

Judy Y. Su, PhD, and Wei-jen Chen, MD

The effects of methylmercury hydroxide (MMH) on the frequency of spontaneously beating atrial-SA node and on the isometric tension of electrically stimulated left atrial (1 Hz) and papillary muscle (0.2 Hz) preparations isolated from rats were investigated. The tissues were also fixed for ultrastructural study at the end of the experiments. We found that MMH, at low doses (0.5 and 2 ppm), increased the frequency of contractions and the isometric tension of isolated atria and decreased the isometric tension of isolated papillary muscles, and at higher doses (>2 ppm), decreased all the above parameters. Morphologically, there were dilatation and swelling of mitochondria and sarcoplasmic reticulum. Occasionally, deposits of amorphous material in the mitochondria and myofibrillary degeneration of myocardial fibers were noted. It is concluded that MMH has direct effect on intracellular organelles (mitochondria, sarcoplasmic reticulum, and myofibril) of myocardial tissue and induces functional changes of atria and papillary muscles. (Am J Pathol 95:753–764, 1979)

THE EFFECTS OF ORGANIC mercurial compounds, especially methylmercury, on the central nervous system are well known.[1-3] The toxicity of methylmercury to lysosomes and mitochondria of cells of the kidney, liver, and intestine have also been reported.[4-7] However, there is very little information about the effects of methylmercury on the cardiovascular system. Only a few scattered reports [8,9] of cardiovascular toxicity of mercurial compounds appeared in the literature. Results from USSR [9] indicated that workers subjected to prolonged exposure to mercury vapor under industrial conditions have an increased number of cardiac ailments with frequent arterial hypotension. In animal experiments they also observed bradycardia and progressive voltage decrease on EKG. Schmidt and Hayman [8] in animal experiments observed fatty degeneration and necrosis of heart muscle following exposure to organic mercury (ethylmercury acetone). One report suggested myofibrillar degeneration as a subtle myocardiopathic effect of methylmercury.[10] Incidental to other experiments on rats, we have observed that some died a few hours after high dose methylmercury exposure. The myocardial content of mercury was high, which led us to suspect that they may have died from cardiovas-

From the Departments of Anesthesiology and Pathology, School of Medicine, University of Washington, Seattle, Washington.

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Address reprint requests to Judy Y. Su, PhD, Department of Anesthesiology RN-10, School of Medicine, University of Washington, Seattle, WA 98195

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cular collapse. Since methylmercury could exert its effects directly on the heart or indirectly through peripheral vascular system, we have chosen to study the direct *in vitro* effects of methylmercury on the heart by using isolated cardiac tissues to avoid the possible secondary effects on the heart through peripheral vascular changes. The experiment is designed to demonstrate the direct effects of methylmercury on chronotropic and inotropic responses and ultrastructural changes of the isolated atria and papillary muscles from rats.

Materials and Methods

Rats were chosen for this study because abundant information concerning the toxicities of metals has been documented in this species. Adult Sprague–Dawley rats weighing 200–300 g body weight were fasted overnight and killed by cervical dislocation the following morning. The hearts were quickly isolated. The right atrium with sinoatrial node, left atrium, and right ventricular papillary muscle were rapidly dissected and fixed on a Blinks' dual tissue bath [11] containing Krebs' solution oxygenated with 95% O_2–5% CO_2, pH = 7.4 at 30C. The isolated tissue was attached to a clip on one end and to a Statham G7B force transducer on the other. The right atrium was beating spontaneously, and the left atrium and papillary muscles were stimulated (Grass S88 Stimulator) electrically with a square-wave DC impulse of 4 msec duration, and the voltage was 10% above threshold for the papillary muscles at 0.2 Hz and twofold threshold for the left atria at 1 Hz. The resting tensions of the muscles were set at the maximum developed tension that the muscle could develop. The isometric tension of the tissues was recorded on a Gilson 6-channel recorder.

Cumulative doses (0.5 ppm–50 ppm) of methylmercury hydroxide (Ventron, Alfa Products, Beverly, Mass.) were administered into the tissue bath at 5-minute intervals after the preparation reached steady state level of tension (45–60 minutes). Individual doses were carried out up to 60 minutes.

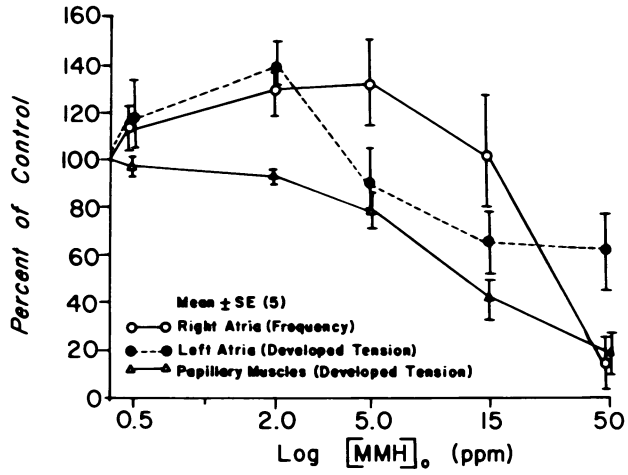
At the end of each experiment, the tissues were removed from the tissue bath and quickly fixed with 3% glutaraldehyde, followed by the buffered osmium tetroxide solution. After dehydration by ethanol, small blocks were embedded in epoxy resin. Ultrathin sections from the blocks were stained with uranyl acetate and lead citrate and examined with an AEI 801 electron microscope.

The frequency of contraction of the right atrium-SA node and the peak developed isometric tensions of the left atrium and the papillary muscle were analyzed. The cross-sectional areas of the papillary muscle were calculated by wet weight/length of the muscle with assumed density of the muscle as one. The significance of the results was tested by the Student t test.[12]

Results

Functional Studies

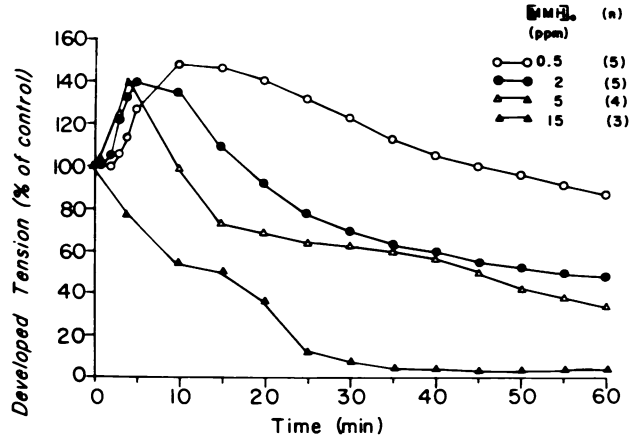
The frequency of contraction of the right atria and developed tension of the left atria and papillary muscles were analyzed with cumulative doses of methylmercury. As shown in Text-figure 1, a biphasic response was observed in both right and left atria. Methylmercury at 0.5 ppm increased both the frequency of contraction (15%) of the right atria and the developed tension (18%) of the left atria, which reached maximum at 2 ppm



TEXT-FIGURE 1—Cumulative dose-response relation. $[MMH]_o$ = Methylmercury hydroxide concentrations in the tissue bath. Mean ± SE (5) = each point represents mean of five preparations and standard errors of the means. For papillary muscles, the average cross-sectional area = 0.9 ± 0.3 sq mm and the control peak developed tension = 1.8 ± 0.2 g/sq mm.

methylmercury (30–40%) and finally decreased as methylmercury concentrations increased. In contrast, a depressive response was the only response of the papillary muscles.

When the time course of the methylmercury effects on the isolated cardiac tissues was studied, a biphasic response was again observed only in the atrial tissues (Text-figure 2). At concentration of 0.5, 2, or 5 ppm, there was an initial increase in developed tension and frequency of contractions (140–150% of control) which reached maximum at about the fifth to tenth minute and declined gradually thereafter. At higher concentrations (> 15 ppm), only depressive response was noted (Text-figure 2). The time course of methylmercury effects on isolated papillary muscle is shown in Text-figure 3. Again, only a monophasic response was observed throughout the



TEXT-FIGURE 2—Time course of methylmercury hydroxide effect on developed tension of isolated left atria. n = numbers of preparations.

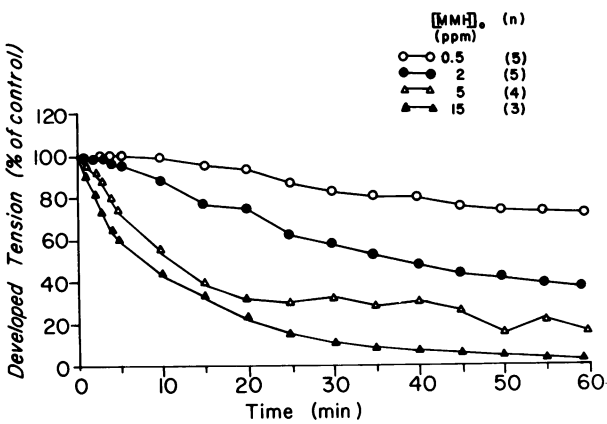
dose range. The rise or decline of developed tension became greater as the methylmercury concentrations increased, as seen in both Text-figures 2 and 3.

Beta adrenergic blocking agent 10^{-6} g/ml propranolol was used to test the possibility that catecholamines were released by low methylmercury concentrations, resulting in increases in frequency and tension of the isolated atrial tissues. The tissues were equilibrated with propranolol for 30 minutes before the administration of methylmercury. We found that propranolol did not block the stimulatory effects of methylmercury on the isolated atria (Text-figures 4A and B). Thus, the stimulatory effects of methylmercury were not due to the release of catecholamines from sympathetic nerve endings.

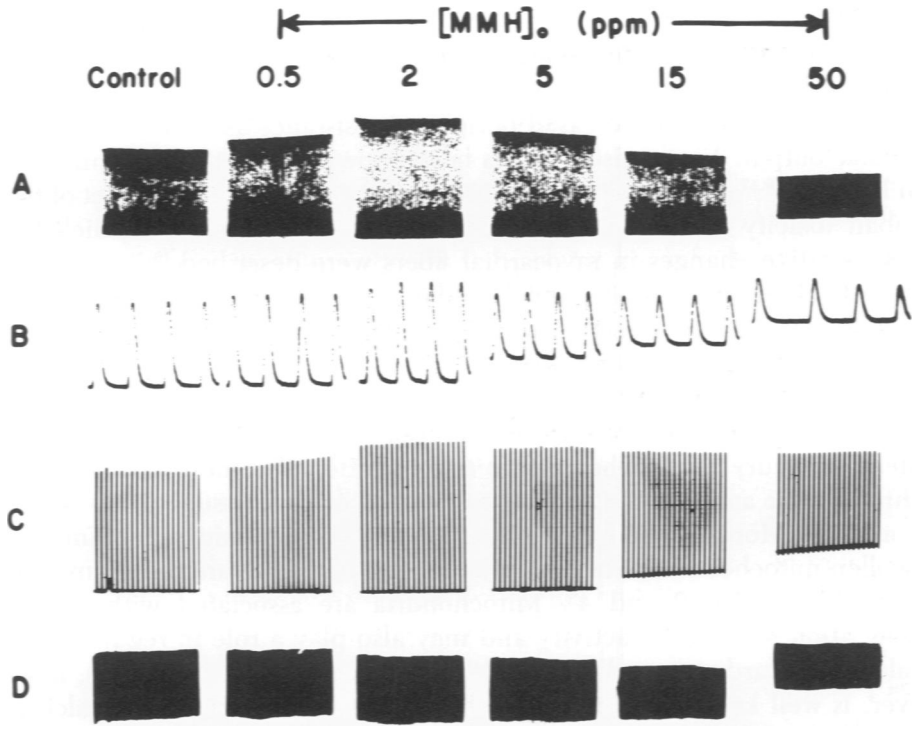
A possible frequency-dependent stimulatory effect of methylmercury was tested by reversing the frequency of stimulus (1 Hz for papillary muscles and 0.2 Hz for left atria). We found that again methylmercury increased the isometric tensions of the left atria but not that of the papillary muscles (Text-figures 4C and D). Thus, the differential stimulatory effects of methylmercury on atrial over papillary muscles were not due to differences in the frequency of stimuli.

Morphologic Changes

The light and electron microscopic appearances of control tissues in Krebs' solution for 50 minutes without methylmercury (right atria were spontaneously beating and left atria and papillary muscles were electrically stimulated) are normal. Figure 1 is a representative section from the left atrium showing normal cellular organelles. When incubated *in vitro* in methylmercury medium, both the atrial tissue and the papillary muscle



TEXT-FIGURE 3—Time course of methylmercury hydroxide effect on developed tension of isolated papillary muscle. n = numbers of preparations.



TEXT-FIGURE 4—Typical tracings for the responses observed after beta blockade (A and B) and after reversed frequency of stimulation (C and D). The tracings were collected at fifth minute after each dose. A—Left atrium stimulated at 1 Hz, paper speed = 10 mm/min. B—Right atrium, paper speed = 25 mm/sec. C—Left atrium stimulated at 0.2 Hz, paper speed = 15 mm/min. D—Papillary muscle stimulated at 1 Hz, paper speed = 15 mm/min. A and B were pretreated with 10 ^{6}g/ml propranolol for 30 minutes, and the changes after propranolol were less than 5%.

show a variable amount of ultrastructural change. In most cases, the tissue was fixed with glutaraldehyde after incubation in 50 ppm Hg for 30 minutes because the rat's blood mercury level usually was about 50 ppm at the time of death in our *in vivo* experiments (Chen and Mottet, unpublished observation). However, ultrastructural changes were also seen at lower mercury concentrations (0.5, 2, 5, 15 ppm, etc.). Dilatation and swelling of mitochondria with disruption of cristae (Figure 2) were found. Occasionally, dense amorphous deposits were observed in the mitochondrial matrix (Figure 3). Dilatation of the sarcoplasmic reticulum with focal disruption of the membrane was also noted (Figure 4). In the papillary muscle, similar morphologic changes were also observed. In addition, cross banding (Figure 5) structurally resembling myofibrillary degeneration [13] was frequently observed. Ultrastructurally, these bandings are composed of aggregates of disorganized myocardial fibrils (Figure 6).

Discussion

The toxic effects of some metals in the cardiovascular system have been described. For example, cadmium [14,15] has been shown to produce diastolic hypertension by increased peripheral resistance as well as increased cardiac output. Lead poisoning has been known to affect cardiac function in humans,[16,17] and it induces cardiomyopathy in mice.[18] One aspect of the cobalt toxicity is the so called "Beer drinker's syndrome" in which the degenerative changes in myocardial fibers were described.[19-21] All these clinical observations and animal studies pointed to the fact that some metals may have a direct toxic effect on the myocardium, though the molecular mechanism is not known.

Experiments of the effects of mercury on the cardiovascular system are even more scarce. The results of this study show a direct toxic effect of methylmercury on the heart *in vitro*. Functionally, there are negative chronotropic and inotropic effects on isolated cardiac tissues (Text-figures 1 and 3). Morphologically, a dilatation of sarcoplasmic reticulum and swollen mitochondria with disruption or loss of cristae are the prominent features (Figures 2 and 4). Mitochondria are associated with energy generation for cellular activity and may also play a role in regulation of calcium in cardiac muscle contraction.[22,23] Sarcoplasmic reticulum, however, is well known for its role in the regulation of intracellular calcium during normal contractile process.[24] How mercurial compounds produce damage to the muscle cells is not clear. It is known that mercurials are one of the sulfhydryl inhibitors. The sulfhydryl group is one of the most reactive and ubiquitous ligands in biologic systems which are involved in cell membrane structure and function.[25] The interaction of organic mercurials with reactive sulfhydryls results in changes in specific transport and permeability systems.[25] It is well known that calcium plays an important role in the excitation–contraction coupling. The mechanisms of methylmercury-induced myocardial depression, which are listed as follows, could be due to an inhibition of one or more steps involved in the contractile process: 1) an inhibition of Na^+-K^+ ATPase,[26] 2) an inhibition of Ca^{2+}-Mg^{2+} ATPase and Ca^{2+} transport through the sarcoplasmic reticulum,[27] 3) an inhibition of enzymes associated with oxidative phosphorylation in mitochondria,[28] 4) an inhibition of the contractile apparatus (myosin ATPase, etc.),[29] 5) removal of myoplasmic free calcium by calcium precipitation in the mitochondria. The depressant effects of the methylmercury on the isolated cardiac tissues appear related closely to the changes in membrane structures and myofibrillar organizations from our study.

The observations of positive chronotropic and inotropic effects of

methylmercury on the isolated atrial tissues at lower concentrations (0.5 and 2 ppm, Text-figure 1) or initial 5 minutes (Text-figure 2) were described. These stimulatory responses of the isolated rat atria were neither frequency-dependent nor mediated through catecholamine release. A similar positive inotropic response of isolated atria caused by various mercurials has also been reported.[26,30] The mechanisms of the stimulatory effects of methylmercury on the atrial tissues are not known. At low concentrations, methylmercury may increase membrane permeability. An increased Ca^{2+} flux through the sarcolemma, sarcoplasmic reticulum, or even the mitochondria could increase the myoplasmic free calcium concentration resulting in increased chronotropic and inotropic responses. Scott et al [31] and Brierley et al [32] have reported that low concentrations of mercurial reagents *in vitro* increase permeability of isolated heart mitochondria, ATP-dependent uptake of K^+, and ATPase activity. However, ionophoric and hydrolytic function of the (Ca^{++} -Mg^{++})-ATPase of sarcoplasmic reticulum were not affected by low concentrations of methylmercury.[27] It would be important to study the localization of mercury and ATPase activity at the ultrastructural level. Currently, this study is under way in our laboratory.

Irrespective of the mechanisms of the stimulatory effects of methylmercury on the isolated atria, it is interesting to note that there was no stimulatory response in papillary muscles. There are no reports of qualitative differences in function and ultrastructure between atria and papillary muscle. Whether the difference in response we observed is caused by a different topographical arrangement of membrane thiol groups or some other factor remains unknown. Because of the crucial importance of trace metals on the molecular mechanisms of cardiac muscle activity, further investigations are needed to fully understand their effects on cardiac pathologic processes.

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[*Illustrations follow*]

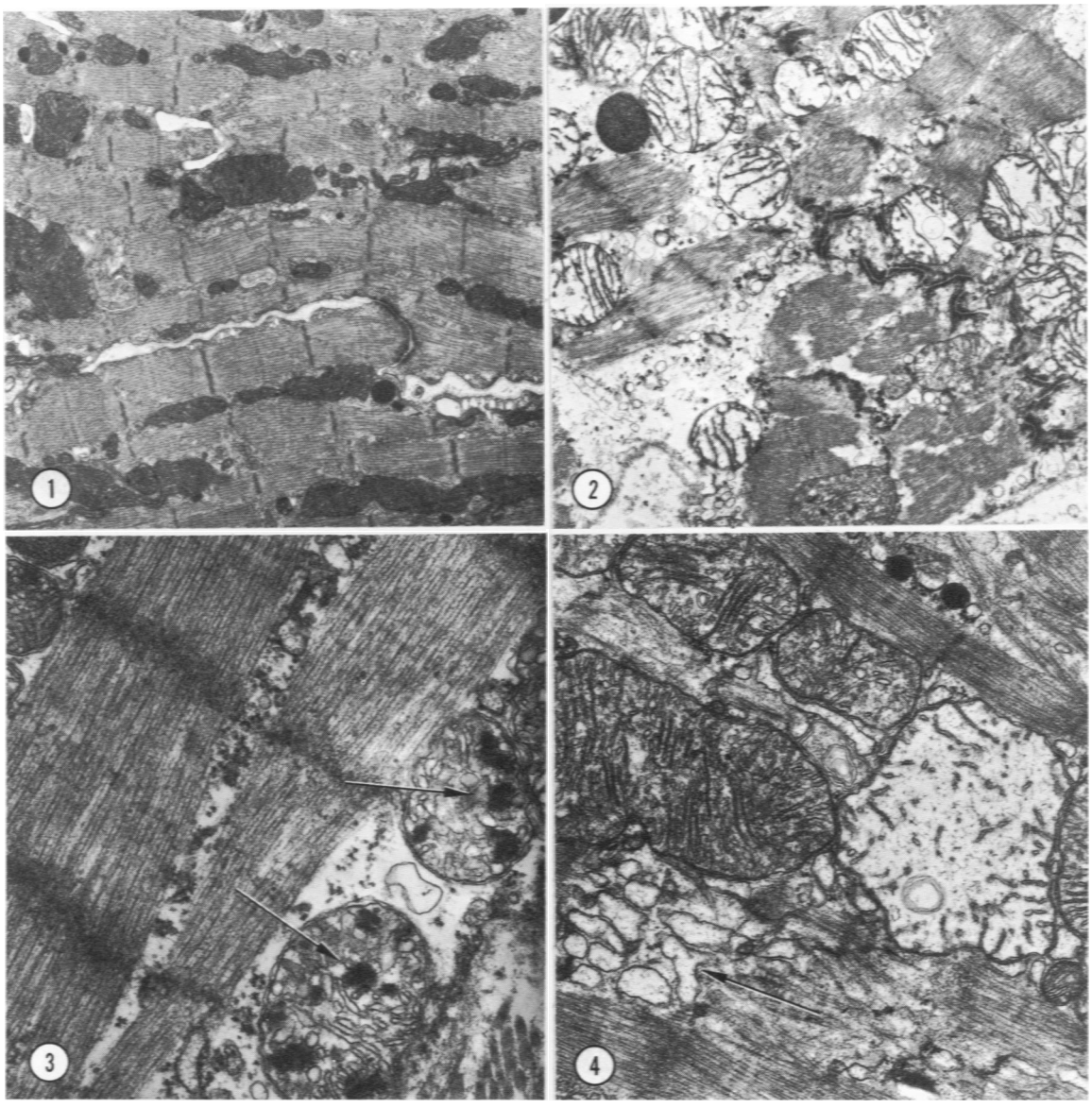

Figure 1—Left atrial tissue electrically stimulated for 50 minutes in Krebs' solution. Ultrastructural picture shown is normal. (× 6300) **Figure 2**—Right ventricular papillary muscle electrically stimulated for 15 minutes in Krebs' solution containing 0.5 ppm methylmercury hydroxide. Swollen mitochondria with disrupted cristae are present. Focal interstitial edema is also noted. (× 6300) **Figure 3**—Left atrial tissue electrically stimulated for 15 minutes in Krebs' solution containing 50 ppm methylmercury hydroxide. There are many dense amorphous material in the mitochondria (*arrows*). Myocardial fibrils appear normal. (× 25,000) **Figure 4**—Same preparation as Figure 3. Dilatation of sarcoplasmic reticulum is evident (*arrow*). One of the mitochondria is swollen with disruption of cristae. (× 10,000) (Figures 1-4, photographic reduction of 14%)

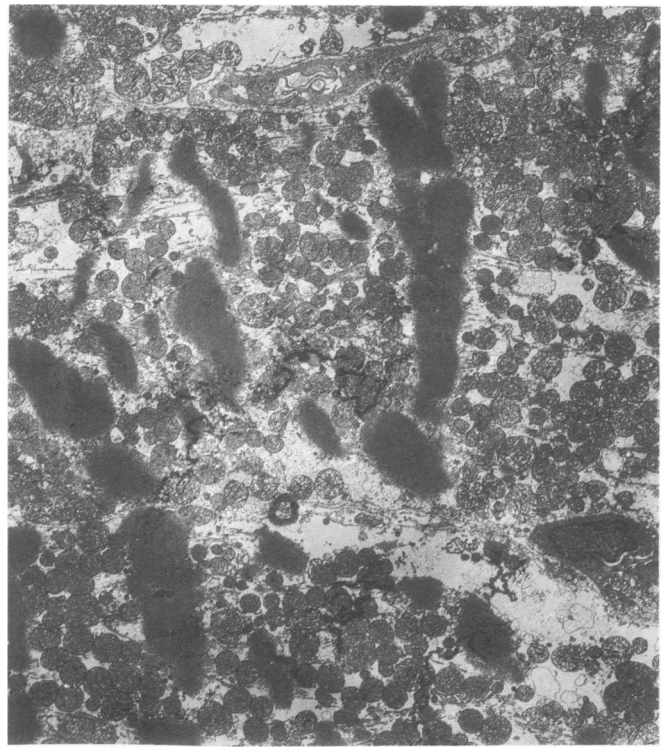

Figure 5—Right ventricular papillary muscle electrically stimulated for 50 minutes in Krebs' solution containing 0.5 ppm methylmercury hydroxide. Multiple cross bandings are present. (× 1600)

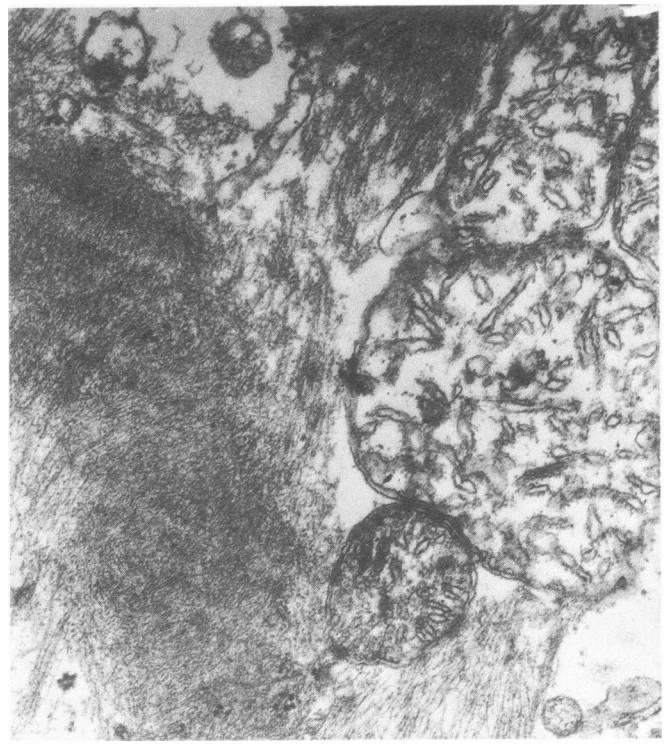

Figure 6—Same preparation as Figure 5. Higher magnification of one of the cross bandings reveals aggregates of disorganized myocardial fibrils. Adjacent mitochondria show various degree of swelling. (× 25,000)